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EFFECTS OF CHLORINE DIOXIDE ON THE NEUROBEHAVIORAL DEVELOPMENT OF RATS

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16. ABSTRACT

Since (1) thyroid function is intimately involved with enurobehavioral development and (2) chlorine dioxide has been shown to disrupt thyroid function, this study was designed to evaluate the effects of chlorine dioxide exposure on selected rat behavioral and brain growth parameters. The data from this study indicate that rat pups that are exposed to chlorine dioxide pre- and post-natally, or exposed only post natally, manifest behavioral changes as well as slower brain growth rates that are consistent with effects caused by depressed thyroid function.

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INTRODUCTION

The production of carcinogenic trihalomethanes[1,2] in drinking water disinfected with chlorine is cause for concern and has led to a search for an alternative drinking water disinfectant. One such alternative is chlorine dioxide (ClO_2). The potential toxicity of ClO_2 and its metabolites (chlorate and chlorite ions) is therefore of interest from a human health perspective. Recent work indicates that exposure to relatively low levels of ClO_2^- (50 mg/L of drinking water) significantly depletes glutathione and increases 2,3-diphosphoglycerate in the red blood cells of rats.[3] Abdel-Rahman et al.[4] found similar results with ClO_2 and ClO_3^-. Suh et al.[5] found that ClO_2 exposure (100 mg/L in drinking water) resulted in depressed DNA synthesis in the testis and intestinal epithelium of rats. African green monkeys (Cercopitheous aethiops) exhibited reduced levels of thyroid metabolism as a result of exposure to 100 ppm of ClO_2 administered in their drinking water (9 mg/kg/day)[6].

The potential role of chlorine dioxide as an anti-thyroid agent has recently been tested by Orme et al.[7] Exposure to ClO_2 either indirectly, via the dam's drinking water (100 ppm) from 14 days prior to

breeding until the pups reached 21 days of age, or directly, by oral gavage from 5 through 20 days of age (14 mg/kg/day) resulted in significantly depressed levels of serum thyroxine (T_4) in 21-day old pups.

Since ClO_2 appears to exert an anti-thyroid effect[7] and since thyroid function is intimately involved with the control of neurobehavioral development[8,9,10], the current study was designed to evaluate the effects of ClO_2 exposure on selected behavioral and brain growth processes. In particular, we report here the effects of ClO_2 exposure on the development of locomotor activity and exploratory behavior, and on the cell numbers and densities in cerebellum and forebrain of rats.

MATERIALS AND METHODS

Exposure Paradigms

Indirect exposure

Rat pups were derived from Sprague Dawley dams which had received 100 ppm ClO_2 in their drinking water from 14 days prior to breeding until the pups were weaned at 21 days of age.

Direct exposure

Rat pups derived from unexposed dams were given a daily dose of 14 mg/kg of ClO_2 in water from age 5 to 20 days postpartum. Control pups received an appropriate amount of distilled water.

Dam food and water consumption as well as dam and pup body weights were monitored weekly for both the indirect and direct exposure groups.

Behavioral Measures

Locomotor Activity

Two different measures were used: (1) A home cage apparatus first described by Croften et al.[11] was used by Orme et al.[7] to evaluate the development of locomotor activity in young rat pups that were exposed to ClO_2 indirectly (via their dams) or directly (by oral gavage) from 5 through 20 days of age. Individual dams and their litters were placed in cages to which the dam was restricted. The activity of the litter of pups was measured when the pups crossed into a small compartment via small connecting holes and interrupted the path of an infrared photobeam that transected the smaller compartment. The unit of measure in this system was the activity of the litter, which was monitored continuously by a computer. The data from the activity cages were

analyzed by analysis of variance procedures.

(2) A residential cage system[12] was used to monitor feeding, drinking and running wheel activity of individual rats from 50 through 60 days of age. The data were collected continuously for 10 days and were analyzed by repeated measures analyses of variance.

Exploratory Behavior

The behavior of 28-day and 60-day old rats was examined in an open-field apparatus during a 15-minute sampling period. Specifically, exploratory behavior was evaluated by monitoring the activity of each rat in a small alcove provided with small openings which served to stimulate investigatory behavior. Infrared photobeams traversed each opening and a computer monitored the duration and the frequency with which the beams were broken. One way analyses of variance and Kruskal-Wallace tests were used to analyze the data.

Biochemical Measures

At either 11 or 21 days postpartum, rats from each exposure paradigm were decapitated. The cerebellum (minus the paraflocculi) and the forebrain (minus olfactory lobes) were quickly removed, weighed on a Mettler PC 180 balance, and frozen at -80° C.

Individual cerebella and forebrains were homogenized in deionized water (Milli-Q Reagent Water System). For the DNA analyses, duplicate 1 mL samples of the homogenates were precipitated with 1 mL cold trichloroacetic acid (TCA). DNA was extracted from the precipitates by hydrolysis in 5% TCA for 15 min at 90° C[13]. Following centrifugation, supernatant fluids were assayed for deoxypentose content with the diphenylamine reagent of Burton[14]. DNA concentrations were estimated by reference to standards prepared from salmon testis DNA (Type III, Sigma). A value of 6.2 pg of DNA per diploid rat nucleus was used for calculating cell numbers[15].

RESULTS

Indirect Exposure Group

Behavior

There were no significant differences in body weight of the dams exposed to 100 ppm of ClO_2 in their drinking water, nor in the body weights of pups born to these dams, as compared to appropriate control animals.

We found that the locomotor activity of the developing pups (10 through 20 days of age) exposed to 100 ppm ClO_2 via their dam was consistently lower than

control pups when tested in the home cage[7]. These differences, however, were not statistically different from the controls ($p = 0.08$; N = 13 litters of controls, N = 16 litters of ClO_2 exposed rats). In terms of exploratory behavior these animals were not significantly different from controls when tested at 28 days of age. However, at 60 days of age they exhibited significantly depressed levels of exploration, as compared to controls ($p < 0.05$ by ANOVA; N = 12 for controls, N = 11 for ClO_2 group).

Body and Brain weights (21-day old pups)

No significant differences were detected in the body weights of exposed versus control animals. Exposure to ClO_2, however, resulted in a significant decrease in the mean whole brain weight ($p < 0.05$, ClO_2 group = 1.39 gm, N = 14; controls = 1.43 gm, N = 15). This decrease is primarily accounted for by a reduction in cerebellar weight ($p = 0.0001$, ClO_2 group = 0.170 gm, N = 14; controls = 0.186 gm, N = 15). Forebrain weights did not differ.

DNA Content (21-day old pups)

The total DNA content of cerebella was significantly lower in ClO_2-exposed animals (938 ug, N = 12) than in controls (1008 ug, N = 12; $p < 0.005$). The difference

of 70 ug represents a deficit of 11-12 x 10^4 in the total number of cells (7.4% reduction; Fig. 1). However, the density of cells in cerebella of ClO_2-exposed animals, based on DNA per gram of tissue, did not differ from controls. The total number of cells and cells per gram of forebrain tissue were the same in exposed (N = 10) and control animals (N = 10; Fig. 1).

Direct Exposure Group

Behavior

Orme et al.[7] found that at 14 days of age the pups directly exposed to ClO_2 by gavage weighed significantly less than control pups (20 gm vs 24 gm, respectively). By 21 days of age the difference between the ClO_2 and control animals was even more marked (31 gm for ClO_2 and 46 gm for control animals) and represented a significant effect ($p < 0.05$).

The pups directly exposed to ClO_2 showed a significant depression in total activity for days 18 and 19 postpartum in the home cage apparatus ($p < 0.05$, N = 18 litters for ClO_2 exposed group and 15 for controls)[7]. Animals randomly selected from these two groups were placed in residential cages for 10 days at 50 days of age. The ClO_2 treated rats exhibited significantly depressed wheel running activity during the night (Fig. 2; $p < 0.05$; N = 7 for ClO_2 treated rats,

N = 8 for control rats). Significant treatment effects on drinking behavior were also detected between the ClO_2 and control rats ($p < 0.05$). Although the ClO_2 treated rats were more active at the water bottle than were the controls, there were no significant differences in water consumption. No treatment differences were detected in feeding behavior or food consumption.

At 28 days of age the ClO_2 treated animals exhibited significantly depressed exploratory behavior as compared to controls ($p < 0.05$; N = 15 for both groups). Littermates of these animals exhibited no significant differences in exploratory behavior when tested at 60 days of age.

Body and Brain Weights (11-day old pups)

The body weights of animals gavaged with ClO_2 tended to be lower than those of controls; the difference in mean body weights approached significance ($p = 0.08$; ClO_2 group = 21.8 gm, N = 8; controls = 24.3 gm, N = 9). In general, the whole brain and brain part weights also tended to be lower in the exposed animals than in the control animals; in the whole brain and cerebellum, the differences approached significance ($p = 0.06$).

Body and Brain Weights (21-day old pups)

The mean body weight of animals treated with ClO_2 (38.2 gm, N=10) was significantly depressed compared to controls (48.6 gm, N = 11; p = 0.0001). The deleterious effect of intubated ClO_2 on body growth is also evident in the significant 12% reduction in the weight of brain tissue of exposed animals. When the mean weights of whole brains and brain parts are adjusted for the differences in body weight (through covariance analysis), the whole brain and forebrain weights of intubated animals are still significantly lower than controls whereas the cerebellum weights of these two groups do not differ.

DNA Content (11-day old pups)

Compared to control animals, the total DNA content of both the cerebellum (ClO_2 group = 476 ug, N = 8; controls = 578 ug, N = 8; $p < 0.004$) and forebrain (ClO_2 group = 728 ug, N = 6; controls = 800 ug, N = 9; p = 0.01) of treated animals was significantly reduced. Intubation of ClO_2 from day 5 through day 11 of postnatal development thus reduces the total cell number in cerebella by more than 16×10^6 cells (17.6% reduction) and in the forebrain by 12×10^6 cells (9.3% reduction; Fig. 3). The number of cells per gram of tissue was not affected.

DNA Content (21-day old pups)

No significant treatment effect on the DNA content of the cerebellum was found. The total DNA in forebrains of treated animals (848 ug, N = 10) was significantly lower than in control animals (937 ug, N = 10; p = 0.0001). The forebrains of treated animals therefore contained 14 x 10^6 fewer cells than forebrains of controls for a 9.3% reducton. No differences, however, were noted in the number of cells per gram of tissue.

DISCUSSION

These data indicate that the rat pups exposed to ClO_2 both prenatally and postnatally until 21 days of age, and those exposed postnatally from 5 through 20 days of age, exhibited behavioral deficits and depressed brain growth consistent with effects produced by depressed thyroid function. Neonatal hypothyroidism results in depressed thyroid hormone levels and is characterized by a delay in age of eye opening, decreased body, whole brain, and cerebellum weights, and decreased activity[8,14,17,18,19]. Both exposure groups in the current study exhibited delays in the development of locomotor activity, as measured in the home cage apparatus and, in the case of the rats exposed to ClO_2 by oral

gavage, this depressed locomotor activity persisted through at least 60 days of age (Fig. 3). To date, only directly exposed rats have been evaluated for long term locomotor activity effects so it is not known if the indirectly exposed animals will continue to exhibit depressed locomotor activity at older ages. However, rats exposed to ClO_2 indirectly do show depressed exploratory behavior at 60 days of age. Littermates of both exposure groups exhibited significantly depressed serum thyroxine levels at 21 days of age and the indirectly exposed group exhibited significantly elevated triiodothyronine levels[7]. The behavioral data are consistant with an explanation based on an anti-thyroid effect of ClO_2.

The whole brain weight and the total number of cells, based on total DNA measurements, was lower in brains of animals from both exposure groups than in controls; no differences in cell packing density were noted, however. The cerebellum appears to be the primary target in the indirectly exposed animals. Although the weight and DNA content of the cerebellum were lower in the directly exposed pups at 11 days of age than in controls, no differences were noted in 21-day old pups; the most persistent effect in this exposure group appeared to be on the forebrain.

A decreased rate of cell acquisition and a prolonged period of cell proliferation are observed in the hypothyroid cerebellum[8]. Thus, the time of onset of

neuronal differentiation (marked by termination of cell proliferation) may be delayed. A reduction in the number of functional cerebellar neurons and an increase in the number of glial cells may occur as a consequence[20]. Lauder[8] concludes that the thyroxine plays an important role in the timing, rate, and quanitity of cell proliferation. Although opposing views are held relative to the influence of thyroxine on brain development during the fetal period[9,21], there is general agreement that deprivation of thyroid hormone during a critical period of postnatal development can lead to defects in the development of some CNS components, particularly the cerebellum. Depressed levels of thyroxine during early development could therefore have profound and lasting effects on the animal's behavior.

In addition to the lower brain cell numbers and the behavioral deficits in animals from both ClO_2 exposure regimes, the animals exposed only postnatally also had significantly lower body weights.

Our data support the view that ClO_2 exposure may adversely affect thyroid function and that the time of exposure, i.e. prenatal/postnatal vs. postnatal, may result in differential effects. The results clearly indicate that ClO-2 exposure affects neurobehavioral development in the rat and that the effects are complex. Cross-fostering studies are currently in progress to evaluate the effects of prenatal and postnatal exposure to ClO_2 on serum thyroxine levels, brain development,

and long-term behavioral processes in rats.

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REFERENCES

1. "Summary Report: Drinking Water and Health," Safe Drinking Water Committee, Vol. 1 (Washington, D.C.: National Academy of Science, 1977), pp. 715-716.

2. Symons, J. M., J. K. Carswell, R. M. Clark, P. Dorsey, E. E. Geldreich, W. P. Heffernan, J. C. Hoff, O. T. Love, Jr., L. J. McCabe and A. A. Stevens. "Ozone, Chlorine Dioxide, and Chloramines as Alternatives to Cl_2 for Disinfection of Drinking Water: State of the Art," in Proceedings of Second Conference on Water Chlorination: Environmental Impact and Health Effects (Ann Arbor, MI: Ann Arbor Science Publishers, Inc., 1978), pp. 555-560.

3. Heffernan, W. P., G. Guion and R. J. Bull. "Oxidative Damage to the Erythrocyte Induced by Sodium Chloride, in Vivo," J. Environ. Pathol. Toxicol. 2:1487-1499 (1979).

4. Abdel-Rahman, M. S., D. Couri and R. J. Bull. "Kinetics of ClO_2 and Effects of ClO_2, ClO_2^-, and ClO_3^- in Drinking Water on Blood Glutathione and Hemolysis in Rat and Chicken," J. Environ. Pathol. Toxicol. 3:431-449 (1980).

5. Suh, D. H., M. S. Abdel-Rahman and R. J. Bull. "Biochemical Interactions of Chlorine Dioxide and Its Metabolites in Rats," Arch. Environ. Contam. Toxicol. 13:163-169 (1984).

6. Bercz, J. P., L. Jones, L. Garner, D. Murray, A. Ludwig and J. Boston. "Subchronic Toxicity of Chlorine Dioxide and Related Compounds in Drinking Water in the Nonhuman Primate," Environ. Health Perspect. 46:47-55 (1982).

7. Orme, J., D. H. Taylor, R. D. Laurie and R. J. Bull. "Hypothyroid Effects on the Development of Locomotor Activity in Rats," J. Toxicol. Environ. Health (in press).

8. Lauder, J. M. "Effects of Thyroid State on Development of Rat Cerebellar Cortex," in Thyroid Hormones and Brain Development, G. D. Grave, Ed. (New York: Raven Press, 1977), pp. 235-254.

9. Hamburgh, M. "An Analysis of Thyroid Hormone on Development Based on in Vivo and in Vitro Studies," Gen. Comp. Endocrinol. 10:198-213 (1968).

10. Sokoloff, L. "Biochemical Mechanisms of the Action of Thyroid Hormones: Relationship to Their Role in

Brain," in Thyroid Hormones and Brain Development, G. D. Grave, Ed. (New York: Raven Press, 1977), pp. 73-91.

11. Crofton, K. M., D. H. Taylor, R. J. Bull, D. J. Sivulka and S. D. Lutkenhoff. "Developmental Delays in Exploration and Locomotor Activity in Male Rats Exposed to Low Level Lead," Life Sci. 26:823-831 (1980).

12. Doran, S. M. "Modification of Behavioral Activity in Laboratory Rats Exposed to Trimethyltin, Dimethyltin, and Trichlorcethylene," Master's Thesis (Oxford, OH: Miami University, 1983).

13. Schneider, W. C. "Phosphorous Compounds in Animal Tissues. I. Extraction and Estimation of Desoxypentose Nucleic Acid and of Pentose Nucleic Acid," J. Biol. Chem. 161:293-303 (1945).

14. Burton, K. "A Study of the Conditions and Mechanisms of the Diphenylamine Reaction for the Colorimetric Estimation of Deoxyribonucleic Acid," Biochem. J. 62:315-322 (1956).

15. Enesco, M. and C. P. Leblond. "Increase in Cell Numb as a Factor in the Growth of the Organs of the Youn Male Rat," J. Embryol. Exptl. Morphol.

10:530-562 (1962).

16. Eayers, J. "Thyroid and Developing Brain: Anatomical and Behavioral Effects," in Hormones in Development, M. Hamburgh and E. Barrington, Eds. (New York: Appleton-Century-Crofts, 1971), pp. 345-355.

17. Shapiro, S. "Hormonal and Environmental Influences on Rat Brain Development and Behavior," in Brain Development and Behavior, M. Sterman, D. McGinty and A. Adinolfi, Eds. (New York: Academic Press, Inc., 1971), pp. 307-333.

18. Hamburgh, M., L. Mendoza, I. Bennett, P. Krupa, Y. Kim, R. Kahn, K. Hogreff and H. Frankfort. "Some Unresolved Questions in the Brain Thyroid Relationship," in Thyroid Hormones and Brain Development, G. Grave, Ed. (New York: Raven Press, 1977), pp. 49-72.

19. Schalock, R. L., W. J. Brown and R. L. Smith. "Long-Term Effects of Propylthiouracil-Induced Neonatal Hypothyroidism," Devel. Psychobiol. 12(3):187-199 (1979).

20. Nicholson, J. L. and J. Altman. "The Effects of Early Hypo- and Hyperthyroidism on the Development of Rat

Cerebellar Cortex. I. Cell Proliferation and Differentiation," Brain Res. 44:13-23 (1972).

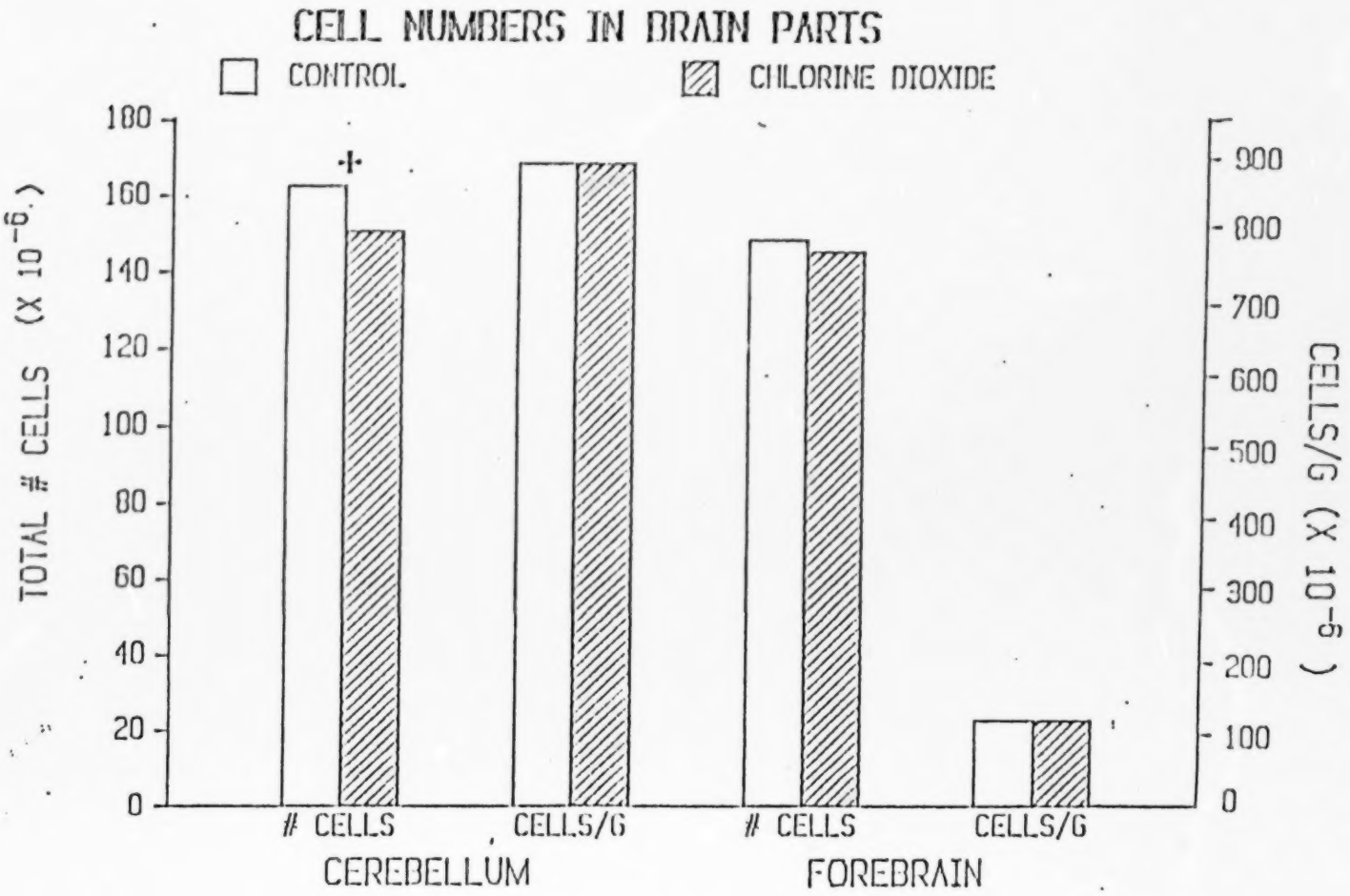
21. Oklund, S. and P. S. Timiras. "Influences of Thyro Levels in Brain Ontogenesis in Vivo and in Vitro," in Thyroid Hormones and Brain Development, G. Grave, Ed. (New York: Raven Press, 1977), pp. 33-45.

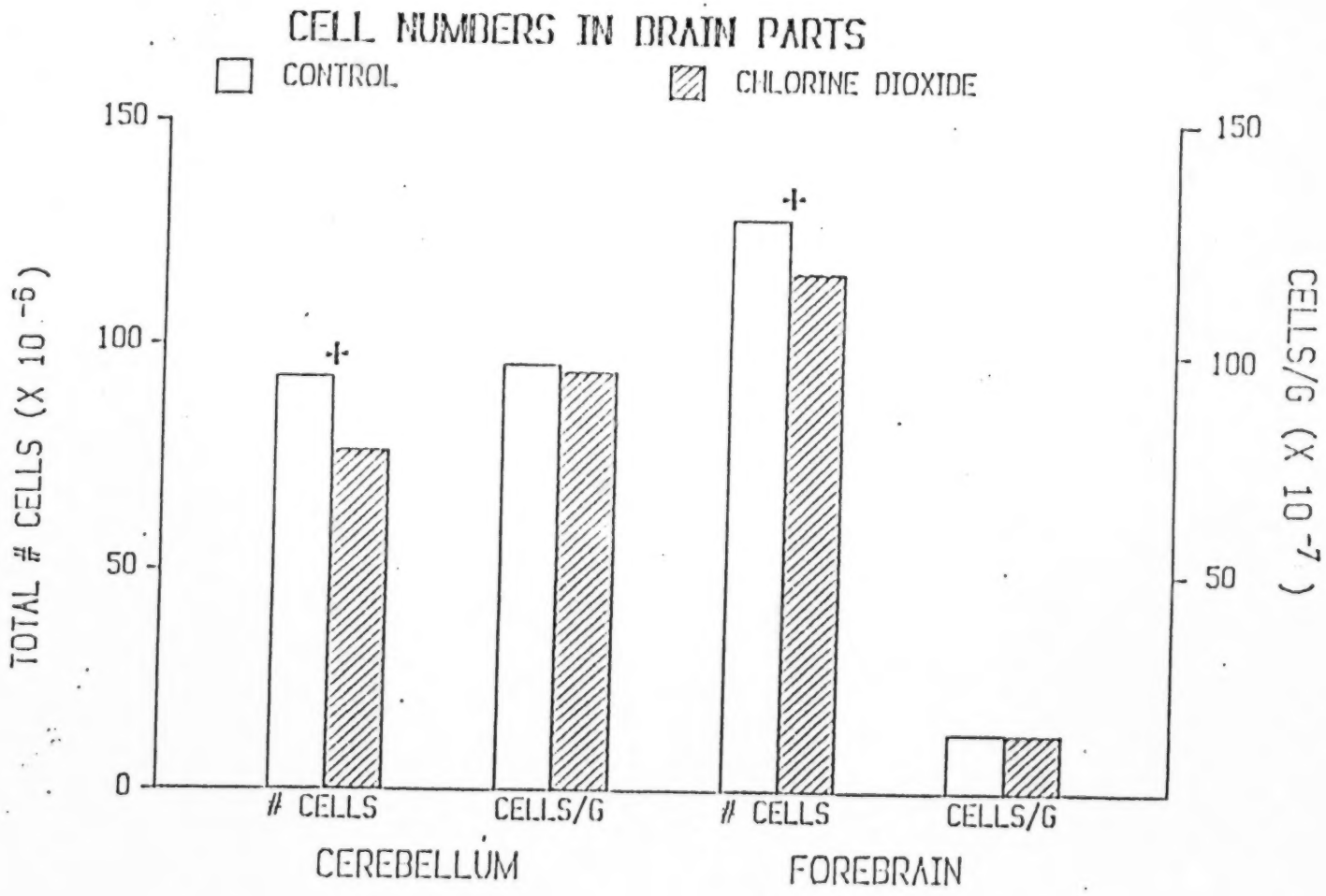
FIGURE LEGENDS

Figure 1. Cell number and cell density in cerebellum and forebrain of 21-day old rats indirectly exposed to ClO_2 prenatally and postnatally. Pairs of means denoted with + are significantly different from each other.

Figure 2. Cell number and cell density in cerebellum and forebrain of 11-day old rats exposed to ClO_2 by oral gavage beginning at 5 days of age. Pairs of means denoted with + are significantly different from each other.

Figure 3. Mean locomotor activity (wheel running) of rats from 50 through 60 days of age that were exposed to ClO_2 by oral gavage from 5 though 20 days of age. Means for all days collapsed to a single 24-hour period.


CELL NUMBERS IN BRAIN PARTS
CONTROL
CHLORINE DIOXIDE
TOTAL # CELLS (X 10⁻⁶)
CELLS/G (X 10⁻⁶)
180
160
140
120
100
80
60
40
20
0
900
800
700
600
500
400
300
200
100
0
CELLS
CELLS/G
CELLS
CELLS/G
CEREBELLUM
FOREBRAIN


CELL NUMBERS IN BRAIN PARTS
CONTROL
CHLORINE DIOXIDE
TOTAL # CELLS (X 10 -6)
CELLS/G (X 10 -7)
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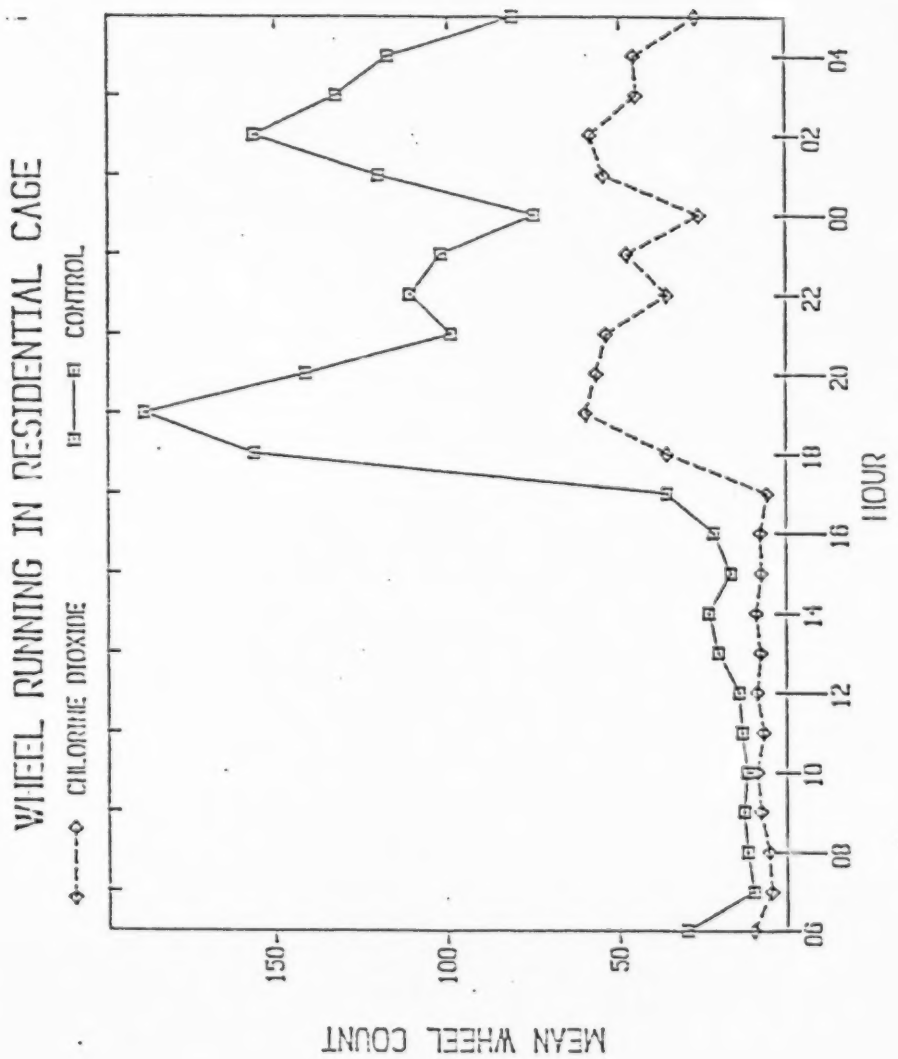
100
50
0
CELLS
CELLS/G
CELLS
CELLS/G
CEREBELLUM
FOREBRAIN


WHEEL RUNNING IN RESIDENTIAL CAGE
CHLORINE DIOXIDE
CONTROL
MEAN WHEEL COUNT
150
100
50
06
08
10
12
14
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22
00
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HOUR